USE OF A BIOMASS OF UNICELLULAR ALGAE FOR HUMAN FEEDING

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Judging from its chemical composition, a biomass of unicellular algae must have high nutritive value [1, 2]. The chemical composition of the biomass depends on cultivation conditions and varies within the following limits: proteins 8.7-88.2, fats 4.5-85.6, carbohydrates 5.7-37.5, ash 3-4% [11]. However, the specific taste and smell of the algal biomass hamper its use for human feeding.

By treatment with alcohols (methanol, butanol, etc.), the algae lose their specific taste, become pale grey in color, and are more palatable than in their untreated condition [6]. /364

The results of degustation tests on products containing algal biomass (Chlorella) have been described in a number of papers [4, 5, 7-9]. Pastries and bread, cooked meat balls, hamburgers, stuffed eggs, Japanese vermicelli soup, ice cream, jellies, and pastes for sandwiches have obtained organoleptic approval. Algae were added to these dishes in the proportion of 5-10%, except for the sandwich paste which contained 75% of algae.

American workers [14] consider that the specific taste of algae is less perceptible in confections such as gingerbread and chocolate cookies or cakes.

Tamura and co-workers [14] determined the assimilation of protein of unicellular algae included in the composition of cakes used in the diet to the extent of 21-23 g (dry weight) daily. They found that algal proteins are assimilated less readily than rice proteins, but the rate of assimilation of fats and carbohydrates was not reduced [15].

Hayami and co-workers [5] tested the assimilation of algal biomass, blanched and decolorized with methanol and given for four days to five women whose diet included 30 g dried algae. Assimilation of protein of the decolorized biomass was greater (86.3%) than that of untreated biomass (74.8%).

Miyoshi [10] included about 30 g algae in the diet of two men. He found that during the period when algae were given in diet the stools of the subjects became dark and fluid, and increased in amount compared with the control period. Compared with initially, the subjects lost a certain amount of weight.

American workers [12] added different quantities of biomass of unicellular algae to human food. The algae were blanched and dried in vacuo. Before the experiment they were autoclaved for 2 h at 160°. Experiments were carried out on five men aged from 18-23 years, with a mean body weight of 71.4 kg (from 63 to 90.5 kg). The investigation continued for 40 days and was divided into 7 periods. The first period (5 days) was the control. The second, third and

fourth periods each lasted six days. During this time dried algae were added to the basic diet at the rate of 10, 20, and 50 g for each subject daily. The 5th and 6th periods each lasted 3 days. During this time algae were added to the basic diet at the rate of 100 and 200 g, respectively. These two periods were separated by an interval of four days when the subjects received the basic diet. /365
The 7th period lasted 2 days and followed immediately after the 6th without an interval. Depending on the dose of added algae, the basic diet was varied to keep its protein content and calorific value constant. In the 7th period, however, the protein content was twice as high as in the control period although the calorific value was slightly lower. This was because the diet consisted only of dried algae (500 g per person per day) with water and a small quantity of fruit syrup added to improve flavor. In the experimental periods the subjects were given gingerbread and chocolate cookies and cakes to which up to 25% of dried algae were added before cooking, and also a mixture of dried algae with cold milk and water in a concentration of 75 g per glass.

On the first days the subjects noticed the strong and unpleasant taste of the algae in the experimental dishes, but after a few days they became accustomed to it. When algal biomass was taken at the rate of 10-20 g daily, the subjects developed flatulence, gases accumulated in the intestine and the abdomen became distended. The stools became dark green in color on the second day of the experimental period and remained colored until algae were excluded from the diet. When the daily dose of dried algae was 50 g, intestinal peristalsis increased and the stools became abundant and dry. On the whole the subjects tolerated addition of up to 100 g algae to the daily ration relatively well. With a dose of 200 g algae in the daily ration, the symptoms mentioned above became more marked. Nausea also appeared. One subject complained of diffuse spasmodic abdominal pain and continuous vomiting. In the 7th period three subjects took part in the experiments. One gave up because he felt unwell, but the other two survived the 2-day period in spite of nausea, weak spasmodic abdominal pain, headache and general malaise. During the whole investigation the subjects lost from 1-2 kg in weight. The daily weight of the stools was doubled during the last two periods of the experiment. The moisture content in the stools fell from 75 to 65%, while their ash, fat, nitrogen and carbohydrate content increased when the subjects received a diet with high concentration of algae, indicating a decrease in its assimilation.

Since a diet with the addition of algae is not a customary one for man, its toxicity was investigated. The subjects were kept under medical observation for six months and no signs of toxicity of the algae were found.

In a leper colony in Venezuela [3] observations were made on patients who received unicellular algae in their diet for between one and three years. Observations were made on 37 persons aged from 8 to 20 years, 26 persons aged from 20-40 years, and 17 aged from 40-70 years. Dry algal biomass (Chlorella) was added to soup at the rate of 10 g for children and 15 g for adults. The pa- /366
tients ate their soup without distaste. They gained in weight and stated that they felt some improvement.

The composition of the intestinal microflora was studied in persons receiving blanched algal biomass (Chlorella) in their diet. Japanese investigators [13] investigated the intestinal microflora of five women aged 21-22 years receiving

30 g blanched algae daily for 10 days in cookies and ice cream. They observed some changes in the content of Escherichia coli and Lactobacillus acidophilus. However, these changes were not consistent in character. American workers [12] report that the E. coli population and the total number of bacteria homogenates of the subjects' stools showed no significant change even when algae was added to the daily diet at the rate of 500 g.

As this survey of the literature shows, a biomass of unicellular algae has no toxic action on man.

Investigations were therefore carried out to study the effect of diets including different quantities of algal biomass (a mixture of Chlorella and Scenedesmus) on some aspects of human metabolism. The maximal quantity of algal biomass which can be included in the human diet was also determined. Three investigations were carried out in which dry biomass was added to the diet at the rate of 50, 100, and 150 g. The algal biomass used was dried lyophilically and had the following chemical composition, expressed relative to the dried substance: protein 51, fat 12, carbohydrates 23, cellulose 5, ash 9%. The biomass of algae used in these investigations was not washed to remove the culture medium.

The chemical composition of the diets was as follows: protein 116-121 g, fat 100-107 g, carbohydrate 460-490 g; calorific value 3360-3440 kcal. Algae were added as jellies prepared in gelatin and agar-agar, a blancmange made with starch, and drinks made with the addition of sugar, raisins, lemon juice, coffee, and vanillin.

The experiments were carried out on 12 volunteers, clinically healthy men aged from 23-39 years, weighing initially 54-100 kg, and 165-184 cm in height.

The subjects performed their normal work.

During the first investigation, when 50 g biomass of unicellular algae was added to the daily diet, three subjects (A., K., and T.) took part, in the second investigation four subjects (M., V., I. and Sh.) received 100 g algae daily, and in the third investigation five subjects (S., Z., E., P. and G.) each received 150 g algae daily. Except for two who had to give up before the end because of their state of health, the subjects received the algal diet for 20-23 days.

Before the beginning and after the end of the investigations, the subjects receiving algae were given a clinical examination. A medical check was applied /367
every day, and the body weight and temperature and the pulse rate determined. Once every ten days, starting with the second investigation, an additional cardiovascular function test together with manual and spinal dynamometry and spirometry was carried out.

Once during the control investigations and twice in the experimental periods the nonprotein nitrogen, urea, ammonia, cholesterol and phospholipids were determined in the venous blood. The 24-hour volume, specific gravity, pH, total nitrogen, urea, ammonia, creatine, creatinine, amino-acid nitrogen, and 17,21-dihydroxy-20-ketocorticosteroids in the urine were determined. Moisture content, total nitrogen, fat, ash, and carbohydrates in the stools were estimated. The subjects noted the volume of water they drank and their 24-hour diuresis. Each subject kept a daily diary in which he recorded how he felt and his assessment of the diet and the daily events.

On the first days of receiving a diet including 50 g algae, subject A, felt dyspeptic symptoms such as flatulence with the smell of algae, nausea, loss of appetite and meteorism. These symptoms disappeared after two days. The dyspeptic manifestations were much less marked in the subjects K. and T. Subsequently all subjects became accustomed to the diet and made no complaints.

All subjects receiving a diet with 100 g algal biomass noticed certain dyspeptic disorders for the first few days (flatulence, meteorism, loss of appetite). Subsequently subjects M., V. and I. felt well until the end of the investigations, although V. and I. had no appetite for dishes containing algae. The subject Sh. had to make an effort in order to eat dishes containing algae before the end of the investigation. No abnormalities of the gastro-intestinal tract were found in this subject.

The subjects responded differently to a diet including 150 g of algal biomass. Subject G. ate dishes containing algae without difficulty throughout the period of investigation, and made no complaints. Subject Z. also tolerated dishes containing algae well, but on the 14th day developed edema of the right eyelids, nose, and frontal region, becoming more severe toward evening, despite an intravenous injection of 10 ml 10% calcium chloride and administration of dimedrol during the afternoon. Next morning the edema spread over the whole face, the right eye could not be opened, and petechial hemorrhages were present on the nose and frontal region. Nevertheless, subject Z.'s general condition was good and he expressed his willingness to continue the investigation. However, he was withdrawn from the investigation and admitted to hospital.

On admission to hospital his general condition was satisfactory. The skin of the face was pale. Marked and firm edema was present in the frontal region, eyelids, nose, cheeks, submandibular region and upper part of the neck on the right side. The fingers were moderately edematous and petechial hemorrhages occurred in the skin of the nose and forehead. Pulse rate 66/min, volume good, blood pressure 110/80 mm Hg. /368

The following treatment was given in hospital: No. 5 diet, cleansing enemas, saline laxatives, dimedrol and rutin orally, ascorbic acid and magnesia intra-muscularly, and calcium chloride intravenously.

On the second day of hospitalization clinical investigations gave the following results: blood formula normal, ESR 3 mm in 1 h, total blood bilirubin 1.37 mg%, cholesterol 206 mg%, thymol test 11 units, mercuric chloride test 2.0, blood sugar 125 mg%. Urine analysis: sp. gr. 1020, reaction acid, protein and sugar absent. Sediment normal. Analysis of stools: test for blood negative. Analysis of gastric juice: total fasting acidity 15, free acidity 0. At the height of digestion, total acidity 25, free acidity 4. Fluoroscopy of the chest and gastro-intestinal tract showed no abnormality. After five days in hospital, subject Z. was transferred to the control diet, which he continued to take until the end of the investigation.

Subject P. reported dyspeptic symptoms (meteorism, loss of appetite) while still on the control diet. After transfer to the experimental diet the dys-

peptic symptoms increased: stools were passed frequently and he disliked the dishes containing algae. He tolerated the drinks containing algae better than the jellies. On the 12th day of receiving the diet with algae, subject P. began to notice slight "cold pricking" on the right hand. On the 15th day a dark band was seen at the edge of the right thumbnail. On the 16th day, the right eyelid and the dorsal surface of the fingers began to itch. The right eyelids were slightly edematous. Because of the appearance of signs of edema, the subject was put back on the control diet. Despite this, next day he noticed slight burning on the dorsal surface of the hand and fingers, especially the right. The skin of the fingers gave an increased reaction to heat (warm water appeared hot). The edema on the face and hands increased. These phenomena persisted for 4 days. On the 5th day the edema of the face and hands diminished. By the 10th day after discontinuing the diet with algae, all these signs had disappeared. Only slight peeling of the skin over the fingers remained.

Throughout the investigation this subject felt well. Apart from the phenomena mentioned above, no other abnormalities affecting the subject's health were observed.

Subject G. developed cyanosis under the nails of the second and third toes and along the medial border of the right foot as far as the base of the big toe 16 days after receiving the experimental diet. The toes were edematous and painful during walking. These phenomena disappeared after two weeks. /369

Subject E. felt a burning sensation in the region of the right big toe 35 days after the end of the investigation. Slight puffiness was found on the medial border of the right foot, including the big toe. The skin of this region was slightly red in color (petechial hemorrhages were seen). These phenomena disappeared after 4 days. Subject S. showed no abnormality affecting his state of health when taking the diet including algae.

Regardless of differences in the content of algae in the diet, all subjects showed an increase in the blood nonprotein nitrogen on the 10th day up to 70-90 mg%, falling to the initial level by the 20th day of the investigation. This can be interpreted as adaptation to the diet containing algae by the 20th day of the investigation. The blood cholesterol of most subjects increased until the 20th day and then fell when the subjects returned to the control diet. In 2 subjects no decrease in the blood cholesterol level was observed 10 days after the diet containing algae was discontinued. The blood cholesterol remained unchanged in one subject throughout the investigation. Since a distinct increase in the blood cholesterol did not take place immediately in these subjects, but on the 20th day after starting to take the algal diet, it can be postulated that these changes were not produced by phytosterols of the algae but by the action of the biomass as a whole on cholesterol metabolism in the body. The changes in the blood concentration of phospholipids in most subjects were identical to changes in the cholesterol level. The other indices investigated were within normal physiological limits. All the subjects remained under medical observation for more than one year and no abnormalities affecting their health were observed.

Pathological changes such as edema and petechial hemorrhages have not been observed by other investigators, probably because of the smaller amount

of algal biomass given in the experimental diets, the shorter periods of investigation, or differences in chemical composition of the diet. These pathological changes, presumably of allergic character, may have been due to components of the culture medium or to other biologically active substances present in the composition of the algae (proteins, carotenoids, the antibiotic chlorellin, and so on).

Further investigations to discover the causes of these abnormalities are in progress at the present time.

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